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ORIGINAL REPORT

Phase I and Pharmacokinetic Study of MS-275, a Histone Deacetylase Inhibitor, in Patients With Advanced and Refractory Solid Tumors or Lymphoma

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Terms in blue are defined in the glossary, found at the end of this issue and online at www.jco.org.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

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ABSTRACT

Purpose
The objective of this study was to define the maximum-tolerated dose (MTD), the recommended phase II dose, the dose-limiting toxicity, and determine the pharmacokinetic (PK) and pharmacodynamic profiles of MS-275.

Patients and Methods
Patients with advanced solid tumors or lymphoma were treated with MS-275 orally initially on a once daily × 28 every 6 weeks (daily) and later on once every-14-days (q14-day) schedules. The starting dose was 2 mg/m^2 and the dose was escalated in three- to six-patient cohorts based on toxicity assessments.

Results
With the daily schedule, the MTD was exceeded at the first dose level. Preliminary PK analysis suggested the half-life of MS-275 in humans was 39 to 80 hours, substantially longer than predicted by preclinical studies. With the q14-day schedule, 28 patients were treated. The MTD was 10 mg/m^2 and dose-limiting toxicities were nausea, vomiting, anorexia, and fatigue. Exposure to MS-275 was dose dependent, suggesting linear PK. Increased histone H3 acetylation in peripheral-blood mononuclear-cells was apparent at all dose levels by immunofluorescence analysis. Ten of 29 patients remained on treatment for ≥ 3 months.

Conclusion
The MS-275 oral formulation on the daily schedule was intolerable at a dose and schedule explored. The q14-day schedule is reasonably well tolerated. Histone deacetylase inhibition was observed in peripheral-blood mononuclear-cells. Based on PK data from the q14-day schedule, a more frequent dosing schedule, weekly × 4, repeated every 6 weeks is presently being evaluated.

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INTRODUCTION

Histone deacetylases (HDACs) regulate gene expression.[1-4] HDAC inhibitors (HDIs) have induced gene activation, cellular differentiation, cell growth arrest, and apoptosis in cancer cells.[4] MS-275 is an orally active synthetic pyridyl carbamate HDI.[5] In the National Cancer Institute's (NCI's) 60 cell-line screen, MS-275 displays a unique pattern of cytotoxicity with potent antiproliferative activity.[6] Microarray analysis suggests MS-275 promotes gene expression favoring growth arrest and differentiation with significantly increased expression of antiproliferation genes such as p21 and transforming growth factor-beta type II receptor, as well as induction of the maturation marker gelsolin.[5-8] In vivo tumor volume reduction was observed in gastric, epidermoid, pancreatic, colon, ovarian, and non–small-cell lung cancer (NSCLC) xenograft models on an oral daily × 28

schedule,[5] as well as in myeloma, promyelocytic leukemia, and small-cell lung cancer models.[6]

Preclinical pharmacology studies indicated that MS-275 peak plasma concentration (T_{max}) was 30 to 40 minutes (administered orally) with a half-life ($T_{1/2}$) of approximately 1 hour, similar in rats, mice, and dogs. Approximately 85% of the drug was bioavailable with oral administration. The dose-limiting toxicity (DLT) was myelosuppression in all species. In an oral daily × 28-day schedule, the maximum tolerated dose (MTD) was 6 mg/m^2 for dogs and 18 mg/m^2 for rats. Adverse events were usually observed during the third and fourth week of dosing. In vitro, human bone marrow sensitivity to MS-275 was similar to rats.[9]

Based on animal data, we conducted a phase I, open-label, single-arm, dose-escalation study in advanced solid tumor and lymphoma patients, with the primary objectives of defining MTD, DLT, and an optimal dose and schedule for a phase II study. Other objectives were to determine safety and tolerability, pharmacokinetic and pharmacodynamic profiles, the ability of MS-275 to affect its target in a surrogate tissue, and antitumor activity. The results of the daily schedule and every-14-day (q14-day) schedule are included in this report.

PATIENTS AND METHODS

Patients

Inclusion criteria were as follows: (1) pathologically confirmed malignancy that was metastatic or unresectable, and for which standard curative or palliative measures did not exist or would likely not be effective; (2) an Eastern Cooperative Oncology Group performance status ≤ 2, with no recent (within 2 months) weight loss of >10% of average body weight; (3) life expectancy greater than 3 months; (4) age ≥ 18 years; (5) leukocytes ≥ 3,000/μL, absolute neutrophil count ≥ 1,500/μL, platelets ≥ 100,000/μL, creatinine within normal limits or measured creatinine clearance ≥ 60 mL/min/1.73m^2, total bilirubin ≤ 1.5 × upper limit of normal, AST /ALT ≤ 2.5 × upper limit of normal, adequate oral intake and serum albumin > 75% of lower limit normal; and (6) able to give written consent, willing to self administer and document the doses of MS 275 as needed, and able to return to NCI for follow-up.

Exclusion criteria were as follows: (1) those who had received prior anticancer therapy (chemotherapy, radiotherapy, vaccines, and hormone therapy with the exception of gonadotropin hormone-releasing hormone agonists) within 4 weeks of study entry (6 weeks for nitrosoureas or mitomycin C, 8 weeks for UCN-01), or those who have not recovered from adverse events (reduced to grade 2 or less) as a result of agents administered more than 4 weeks earlier; (2) known brain metastases; (3) history of allergic reactions attributed to compounds of similar chemical or biologic composition to MS-275; (4) uncontrolled intercurrent illness; (5) pregnant or lactating women; (6) men and women of reproductive potential without adequate contraception; (7) known HIV; (8) gastrointestinal conditions that might predispose for drug intolerability or poor drug absorption; and (9) major surgery within 21 days of study entry, intercurrent radiation, chemotherapy, immunotherapy, or hormonal therapy (except for gonadotropin hormone-releasing hormone agonists).

Dosage and Dose Escalation Scheme

The initial human dosing schedule was daily oral administration for 28 days and 14-day recovery period, constituting a 42-day cycle. MS-275 was administered with food, owing to evidence of enhanced bioavailability from animal studies in the fed state. A starting dose of 2 mg/m^2 (1/10th rat MTD) with an accelerated dose escalation at increments of 100% and single patient per dose level was planned.

Due to unexpected toxicities, the subsequent dosing schedule was changed to once orally every 14 days. Administered in the fed state, the starting dose level was again 2 mg/m^2, using a modified Fibonacci dose escalation scheme (three to six patient cohorts) with a dose escalation increment of 2 mg/m^2 without intrapatient dose escalation.

DLT was defined as first course adverse events ≥ grade 3 nonhematologic or ≥ grade 4 hematologic toxicity. The MTD was defined as one dose level below the dose at which ≥ two of six patients experience DLT.

Dose reduction by one level was applied for the occurrence of either grade 3 nonhematologic toxicity, grade 4 hematologic toxicity, persistent (≥ 2 weeks) grade 2 nonhematologic toxicity, or per the investigator's assessment. For dose level 1, 25%, 50%, and 75% decrease in starting dose was the order of dose reduction. No limitation for the number of dose reductions was chosen.

Safety and Efficacy Measures

At study entry, history, physical examination, laboratory studies (CBC, electrolytes, creatinine, blood urea nitrogen, total and direct bilirubin, ALT, AST, alkaline phosphotase, uric acid, prothrombin time, partial thromboplastin time, and urinalysis), computed tomography scan, and chest x-ray and ECG were performed. Clinical assessments, including a physical examination and adverse event evaluation, were conducted at each follow-up. Adverse events were graded by the NCI Common Toxicity Criteria (version 2.0). Computed tomography scans and staging was performed every 6 weeks for the q14-day schedule. Disease-specific staging techniques, such as bone marrow aspirate and biopsy, flow cytometry, cutaneous lesion photography, or bone scan were used as indicated. Response evaluations used the Response Evaluation Criteria in Solid Tumors[10] and the Cheson criteria[11] for lymphoma. Multiple-gated acquisition (MUGA) scans were obtained on the q14-day schedule at base line, before course 2, and at each restaging. Laboratory studies (CBC with differential, chemistry 20, prothrombin time, and partial thromboplastin time) were performed on days 1, 3, 5, and 7 and repeated weekly. Twenty-four-hour urine clearance, albumin, protein, uric acid, and electrolytes were performed at baseline, and on days 3 and 13.

Pharmacokinetic Studies

Blood samples (6 mL) were collected in sodium heparin tubes at 0, 2, 6, 12, 24, 36, 48, 60, 72, 84, and 96 hours after the first dose. Following initial pharmacokinetic evaluation of data obtained from the first two dose levels, the sampling also included 30 minutes and 1 hour. Samples were immediately centrifuged at 3,000 g for 10 minutes at 4°C and then plasma was divided into two aliquots of at least 1 mL and frozen at −70°C until the time of analysis. Plasma samples were assayed by a specific and sensitive high-performance liquid chromatographic assay with

mass-spectrometric detection.[12] The lower limit of quantitation of this assay is 0.50 ng/mL, with values for precision and accuracy of ≤ 5.58 and ≤ 11.4% relative error, respectively.[12]

Estimates of pharmacokinetic parameters for MS-275 were derived from individual concentration-time data sets by noncompartmental analysis using the software package WinNonlin version 4.0 (Pharsight Corporation, Mountain View, CA). The peak plasma concentrations and the time to peak concentrations were the observed values. The area under the plasma concentration versus time curve (AUC) was calculated using the linear trapezoidal method from time zero to the time of the final quantifiable concentration (AUC_{tf}). The AUC was then extrapolated to infinity (AUC_{inf}) by dividing the last measured concentration by the rate constant of the terminal phase (k), which was determined by linear-regression analysis of the final three or four time points of the log-linear concentration-time plot. The apparent oral clearance of MS-275 (CL/F) was calculated by dividing the administered dose by the observed AUC_{inf} and the $T_{1/2}$ was calculated by dividing 0.693 by k.

Statistical Analysis

Dose proportionality for MS-275 was assessed using a power model (ie, $AUC = \alpha \times dose^{\beta}$) where an ideal proportional model corresponds to $\beta = 1$ (ie, to a model of the form $AUC = \alpha \times dose$) and with the proportionality constant α. Deviations of β from 1 correspond to deviations from ideal dose proportionality. Interindividual differences in pharmacokinetic parameters were assessed by the coefficient of variation (CV), expressed as the ratio of the standard deviation to the observed mean (SD/M). All pharmacokinetic data are presented as mean ± SD except where otherwise indicated. The apparent CL/F and the $T_{1/2}$ were analyzed as a function of the MS-275 dose level using the Kruskal-Wallis' one-way analysis of ranks followed by the Dunn's multiple comparison test for identifying statistically significantly different groups. Variability in parameter estimates for MS-275 between cohorts of patients that did or did not experience DLT was evaluated by a one-sided Mann-Whitney U test for differences in medians after testing for normality and heteroscedasticity. One-way analysis of variance was performed to compare mean values using a two-sided Dunnett's test. Statistical calculations were performed using the Number Cruncher Statistical System 2001 series (J. L. Hintze, Kaysville, UT). The cut-off for statistical significance was considered at $P < .05$.

Pharmacodynamic Analysis

Immunocytochemical analysis of acetylated histone H3 was performed on peripheral-blood mononuclear cells (PBMCs), which were isolated from whole blood by centrifugation on Ficoll-Paque Plus (Amersham, Little Chalfont, United Kingdom), pelleted onto glass slides by cytocentrifugation, fixed in 95% ethanol/5% glacial EDTA for 1 minute and permeabilized with 0.2% Triton X-100 for 10 minutes at room temperature, then nonspecific binding sites were blocked by incubating the cells with 1% bovine serum albumin in phosphate-buffered saline (PBS) for 1 hour at 4°C. Slides were incubated with polyclonal antiacetylated histone H3 antibody (Upstate Biotechnology, Lake Placid, NY) for 1 hour at 4°C, washed two times for 2 minutes with PBS, then incubated at 4°C for 1 hour with Cy3-conjugated goat antirabbit immunoglobulin (Molecular Probes, Eugene, OR), and washed again with PBS. Finally, slides were incubated with 4,6-diamidoino-2-phenylindole (Sigma, St Louis, MO) for 10 minutes at room temperature, rinsed quickly with water, air-dried, mounted using SlowFade (Molecular Probes), and imaged using a Zeiss Axiophot microscope interfaced with a CCD camera (Optronics Engineering, Goleta, CA). Positive controls were prepared by exposing healthy donor PBMCs to MS-275 in vitro. Buffy coats, provided anonymously as a byproduct of whole-blood donations from paid healthy volunteer donors through an international review board–approved protocol, were centrifuged on Ficoll-Paque Plus. Mononuclear cells were depleted of monocytes by adherence to plastic for 2 hours at 37°C and incubated with MS-275 in vitro for various times and at varying drug concentrations. Cells were processed for histone hyperacetylation in the same manner as patient samples. Images of PBMCs stained for acetylated histone H3 were imported into the Openlab image analysis program (Improvision, Coventry, United Kingdom) and histone acetylation levels were assessed using the Openlab quantification software.

RESULTS

General

Between April 5, 2001 and July 29, 2003, 31 patients have enrolled on the study (two on daily and 29 on the q14-day schedule). Thirty of them received MS-275 and were assessable. One patient with melanoma withdrew before receiving treatment owing to a disease complication. All patients (demographics in Table 1) had received prior therapy (median No. of prior treatments = 3): surgery (90%), prior chemotherapy (97%), radiotherapy (50%), and immunotherapy (50%).

Dose Escalation and DLT in Daily and q14-Day Schedule

The dose escalation experience for both the MS-275 daily and the q14-day schedules are summarized in Table 2.

Daily schedule. Two male patients were treated at the initial dose level of 2 mg/m^2 of the daily × 28 schedule. Both experienced DLT before the completion of the first cycle. DLTs observed were abdominal/epigastric pain in one patient, and cardiac arrhythmia (supraventricular tachycardia), elevated AST/ALT, hypotension, hypoalbuminemia, and hypophosphatemia in a second patient. All adverse events resolved within 2 to 3 weeks. Preliminary pharmacokinetic data from our initial two patients suggested that MS-275 had a 30- to 50-times longer half-life in humans than initially predicted from the animal models. This may explain the unforeseen toxicity observed in these two patients during the daily MS-275 schedule. Assessment of histone H3 and H4 acetylation indicated HDAC inhibition occurred after one dose of MS-275. To ensure safety, a q14-day dosing schedule was implemented.

q14-day schedule. A total of 28 patients have been treated on the q14-day schedule. The DLTs of MS-275 on a q14-day schedule were anorexia, nausea, vomiting, and fatigue. The MTD and recommended phase II dose of MS-275 for a q14-day schedule was 10 mg/m^2. As summarized in Table 2, the first patients with first course DLTs

Table 1. Demographics

Characteristic		No. of Patients
Total		31
Age, years		
Median	57	
Range	36-76	
Sex		
Male		19
Female		12
ECOG performance status		
0		7
1		21
2		3
Median		1
Tumor type		
Melanoma		6
Renal cell carcinoma		6
NSCLC		4
Sarcoma		4
Breast		2
Colorectal		2
Lymphoma		2
Cervix		1
Mesothelioma		1
Prostate		1
Small bowel		1
Thyroid		1
No. of prior chemotherapy		
0		1
1		6
2		10
≥ 3		14
Median		3
Range		0-20
No. of prior radiotherapy		
0		16
1		9
2		4
≥ 3		2
No. of prior immunotherapy		
0		16
1		9
2		6

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NSCLC, non–small-cell lung cancer.

were observed at dose level 3 (6 mg/m^2). After five patients tolerated dose level 4 without DLT, dose escalation continued to level 5 (10 mg/m^2). One patient experienced similar DLTs at level 5 as had been seen at level 3. At dose level 6 (12 mg/ m^2), two patients experienced similar DLTs.

First course adverse events observed, either probably or possibly related to MS-275, are summarized in Table 3. There were no MS-275-related first course grade 4 adverse events There was only first course grade 4 adverse event (dyspnea) observed during the study, which occurred at dose level 6 (12 mg/m^2), was considered unrelated to the MS-275, and likely due to progression of metastatic mesothelioma. MS-275-induced fatigue, anorexia, nausea, and vomiting were observed as early as dose level 1 (2 mg/m^2), and all were mild. With dose escalation, intensity of these toxicities gradually increased. Other less frequent drug-related toxicities included taste change, headache, diarrhea, flatulence, bloating, and reflux symptoms. Hematologic toxicities, such as thrombocytopenia and neutropenia, became more apparent at the higher dose levels (Table 3). Anemia was frequently noticed during the first course due to frequent pharmacokinetic and laboratory sampling, not related to MS-275.

Among drug-related biochemical abnormalities observed during the first course, the most frequently observed was hypoalbuminemia. Twenty-four hour urine analysis indicated there is no renal wasting of albumin, protein, or electrolytes. Clinically, no obvious gastrointestinal albumin loss was observed. The hypothesis that MS-275 may trigger inflammatory response, leading to albumin decrease, was examined by evaluating several patients' fibrinogen, C-reactive protein, and ferritin levels at baseline and after receiving MS-275, and no significant changes were found. No change in ACTH, cortisol, progestin, and estrogen was observed in patients who entered higher MS-275 dose levels (8, 10, and 12 mg/m^2) at 0 and 24 hours after the first dose. However, the prealbumin level was decreased after MS-275 administration, suggesting the possibility of production decline.

Symptomatic cardiac adverse events were not observed in patients who received q14-day MS-275. In 184 ECGs performed among 28 patients, there were no statistical or clinical adverse ECG interval (HR, PR, QRS, and QTc) effects observed. There were no ST–T wave changes from the

Table 2. Schedule, Dose Level, and Dose Administration

Dose Level and Schedule	Dose (mg/m^2)	Initial Patient No.	Total No. of Treatment Courses	No. of Patients With First Course DLT	DLTs
Every day × 28/42 days					
1	2	2	2*	2	See text
Every 14 days					
1	2	3	22 (4)	0	0
2	4	3	16 (4)	0	0
3	6	6	51 (8)	1	3†
4	8	5	22 (9)	0	0
5	10	6	30 (8)	1	3†
6	12	5	16 (5)	2	7‡

NOTE. Numbers in parentheses indicate total patients treated at dose level.
Abbreviation: DLT, dose-limiting toxicity.
*Due to DLTs, both patients' treatments were terminated before completing the first course.
†Anorexia, nausea, and vomiting.
‡Anorexia, nausea, vomiting, and fatigue.

Table 3. First Cycle Adverse Events Probably or Possibly Related to MS-275 at all Dose Levels (N = 28)

Adverse Events	All Grades No. of Patients	%	Grade 3
Cardiovascular			
Sinus Tachycardia	1	3	
Hematologic			
Anemia	8	29	
Leucopenia	6	21	
Lymphopenia	5	18	
Neutropenia	7	25	
Thrombocytopenia	10	36	
Gastrointestinal			
Anorexia	10	36	4
Constipation	2	7	
Diarrhea	2	7	
Dyspepsia	6	21	
Flatulence	3	11	
GI other	2	7	
Nausea	18	64	4
Stomatitis	1	4	
Vomiting	11	39	4
Laboratory			
Alkaline phosphatase	1	4	
Bilirubin	4	14	
Creatinine	2	7	
Hyperglycemia	3	11	
Hypermagnesemia	2	7	
Hypoalbuminemia	18	64	
Hypocalcemia	6	21	
Hypokalemia	1	4	
Hyponatremia	7	25	
Urinary electrolyte wasting	3	11	
General			
Allergic reaction	1	4	0
Dehydration	3	11	0
Depression	1	4	0
Fatigue	15	54	1
Fever	1	4	0
Headache	14	50	0
Infection w/o neutropenia	2	7	0
Libido	1	4	0
Middle ear infection	1	4	0
Muscle weakness	1	4	0
Myalgia	1	4	0
Nail changes	1	4	0
Sweating	1	4	0
Taste disturbance	8	29	0
Neuromuscular			
Neurosensory deficits	2	7	
Tremors	1	4	
Pain			
Abdominal pain	2	7	
Chest pain	2	7	
Pain other	1	4	
Pleuretic pain	1	4	
Respiratory			
Cough	1	4	
Rhinitis	1	4	

baseline. Ninety-one MUGA scans were performed. The mean left ventricular ejection fraction (LVEF) was 58.2% ± 1.62 (n = 28) at baseline and 58.7% ± 1.08 (n = 26) at follow-up. Twenty-six of 28 patients had both baseline MUGA and at least one follow-up MUGA. There were no statistically significant LVEF changes detected by the paired t test in these 26 patients ($P = .526$) or per individual dose level ($P = .106$ for 2 mg/m^2; $P = .350$ for 4 mg/m^2; $P = .133$ for 6 mg/m^2; $P = .951$ for 8 mg/m^2; $P = .201$ for 10 mg/m^2; and $P = .834$ for 12 mg/m^2).

A total of 157 courses of MS-275 were administered on the q14-day schedule (Table 2). Some cumulative adverse events caused treatment interruption with repeated MS-275 dosing. For example, grade 1 to 2 adverse events that occurred during early courses may progress to higher grades during later courses, requiring reduction in dose or dosing frequency. The dose reductions were frequent on dose levels higher than 8 mg/m^2, as noted in Figure 1 and Table 4. Frequent cumulative drug-related adverse events observed at or beyond course 2 were: anorexia, nausea, hypoalbuminemia, fatigue, headache, diarrhea, neutropenia, thrombocytopenia, leukopenia, and hypophosphatemia. Table 5 summarizes all drug-related occurring and grade 3 and grade 4 adverse events, occurring with a frequency of > 10% during the second course and beyond. Incidences of dose reduction after the second and subsequent courses of MS-275 are shown in Figure 1 and Table 4. On a q14-day schedule, the lowest doses (2 to 4 mg/m^2) are well tolerated, with ≤ 33% of patients going on to dose reduction, while in the 6 to 10 mg/m^2 dose range, ≥ 50% of patients ultimately required dose reduction. One patient with metastatic NSCLC, who had stable disease at the first restaging, withdrew himself from the study on the seventh day of course 4 and elected to receive standard chemotherapy (docetaxel 50 mg/m^2 every 3weeks). This patient developed a grade 4 neutropenia and leukopenia 8 days after receiving docetaxel, which was 16 days after the course 4 MS-275 dose. Taken together, the data of Tables 4 and 5 and Figure 1 suggest that while 10 mg/m^2 every 14 days was the formal MTD

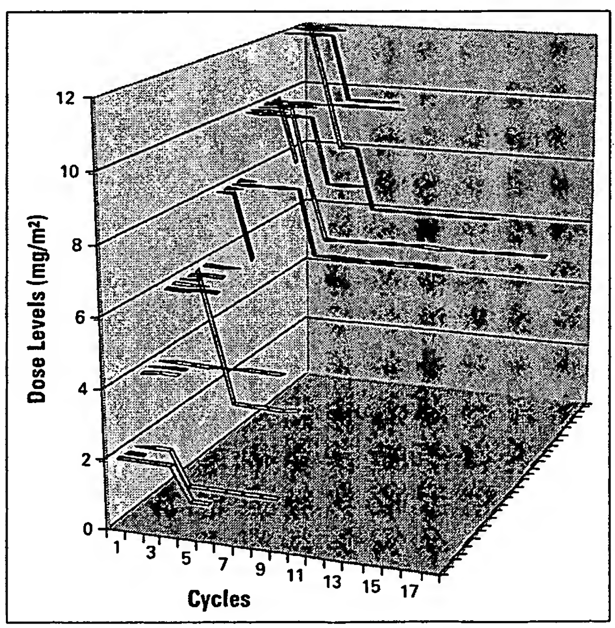

Fig 1. Patient dose reduction on an every-14-day schedule at each dose level. Each line represents a single patient started at their enrolled dose level and all subsequent dose modifications.

Table 4. No. of Patients Who Required Dose Reduction After Course 1

	2 mg/m²	4 mg/m²	6 mg/m²	8 mg/m²	10 mg/m²	12 mg/m²	Total No. of Patients
Course 1	3	3	6	5	6	5	28
Course 2	3	3	5	4	5	3	23
Course 3 or later	2	0	2	2	3	3	12

according to the definition of the protocol, in practice, titration of the tolerated dose to lower doses may be necessary during chronic or more frequent dosing.

With respect to reported HDAC inhibitor–induced immunosuppression, lymphopenia was observed during MS-275 administration. Only three instances of herpes simplex virus–positive stomatitis were found in patients receiving more than one course. A cutaneous T-cell lymphoma patient who had stable disease for over 4 months experienced one episode of herpes zoster recurrence in conjunction with clinical worsening of a skin bacterial infection.

Table 5. Frequent (≥ 10%) Adverse Events Observed During or After Course 2, Probably or Possibly Related to MS-275 (N = 23)

Adverse Events	All Grade: No. of Patients	All Grade: %	Grade 3	Grade 4
Cardiovascular				
Left ventricular ejection fraction	3	13	0	0
Gastrointestinal				
Abdominal pain	5	22	0	0
Anorexia	13	56	1	0
Constipation	3	13	0	0
Diarrhea	8	35	3	0
Dyspepsia	6	26	0	0
Flatulence	3	13	0	0
Nausea	19	83	4	0
Stomatitis	3	13	0	0
Vomiting	7	30	1	0
General				
Arthralgia	4	17	0	0
Chest pain	4	17	0	0
Dehydration	5	22	0	0
Edema	4	17	0	0
Fatigue	23	100	3	0
Fever	5	22	0	0
Headache	12	52	0	0
Myalgia	7	30	0	0
Taste disturbance	10	43	0	0
Urine retention	2	9		
Hematology				
Anemia	6	26	0	0
Leukopenia	8	35	2	1
Lymphopenia	4	17	0	0
Neutropenia	17	74	3	1
Thrombocytopenia	14	61	1	0
Laboratory				
Alkaline phosphatase	3	13	0	0
Creatinine elevation	3	13	0	0
Hypercalcemia	4	17	0	0
Hyperglycemia	5	22	0	0
Hypernatremia	1	4	0	0
Hypoalbuminemia	11	48	1	0
Hypocalcemia	10	43	1	0
Hypomagnesemia	6	26	0	0
Hyponatremia	8	35	2	0
Hypophosphatemia	6	26	4	0
ALT	3	13		
Neuromuscular				
Muscle weakness	3	13		
Neurosensory	3	13		
Respiratory				
Dyspnea	4	22		

Responses

The treatment duration for each patient on the q14-day schedule is depicted in Figure 2. No complete or partial response was observed on the q14-day schedule. Fifteen cases of stable disease were observed with durations of 62 to 309 days. One cervical cancer patient, initially treated at 12 mg/m² had dose reduction thrice, continued on 6 mg/m² every 3 weeks after the fourth course, and sustained a 10-month period of stable disease. One NSCLC patient initially treated at 10 mg/m² also had dose reduction twice, with stable disease for 9 months. Two melanoma patients initially treated at 8 mg/m² and reduced to 6 mg/m² had stable disease for 4 and 5 months, respectively.

Pharmacokinetics

Pharmacokinetic studies were performed in 28 patients, with complete concentration-time profiles available for 27 patients. Figure 3 shows that plasma concentration

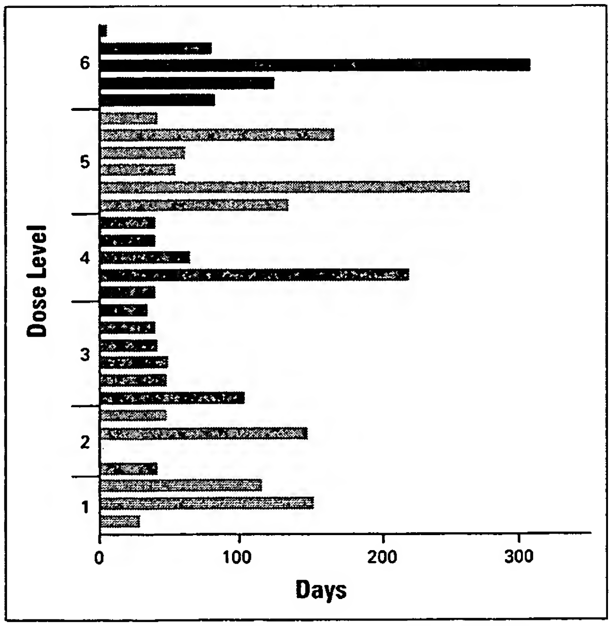

Fig 2. MS-275 treatment duration for patients on an every-14-day schedule. Treatment length ranged from 11 to 309 days. Although no complete response or partial response was observed on this regimen, 15 (52%) of 27 patients had stable disease ranging from 62 to 309 days.

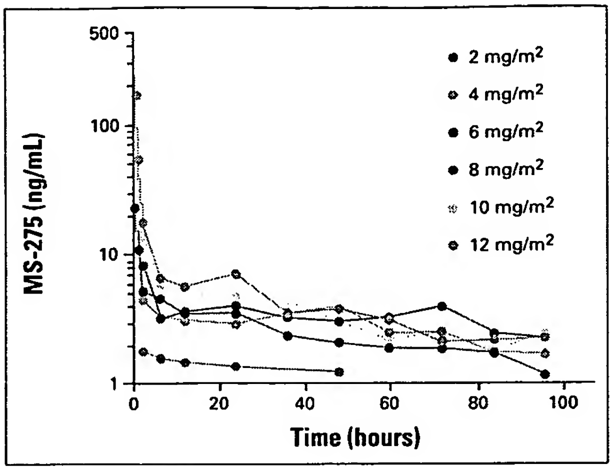

Fig 3. Concentration-time profiles of oral MS-275 at dose levels ranging from 2 to 12 mg/m^2 (n = 27). Data from the same dose levels were grouped and are presented as mean values (symbol) ± SE (error bar). The legend indicates each of the dose levels used.

versus time profiles of MS-275 were very similar at each dose level. The mean noncompartmental pharmacokinetic parameters of MS-275 ranging from 2 to 12 mg/m^2 are summarized in Table 6. Substantial interpatient variability in pharmacokinetic parameters was apparent at any dose level (CV for AUC, up to 53%). Similar variability was apparent in the CL/F (CV = 38.8%), implying varied systemic exposure to MS-275 during drug treatment. Absorption of the drug was highly variable with median T_{max} approaching 2 hours, with slow gastrointestinal uptake of MS-275 resulting in a T_{max} at 24 hours (n = 2), 48 hours (n = 1), and even 60 hours (n = 1), whereas a few patients exhibited T_{max} at 0.5 hours (n = 7), suggesting a rapid absorption and possible underestimation of the extent of drug uptake in these individuals.

Disappearance of MS-275 from the central plasma compartment was characterized by elimination in an apparent bi-exponential fashion, with an overall slow apparent CL/F of 17.4 ± 6.75 L/h/m^2. The estimated apparent terminal disposition half-life was relatively consistent in all patients, exhibiting a mean value of 51.74 ± 21.55 hours (CV = 41.7%). As a result of the slow clearance, MS-275 was detectable even 5 days after initial treatment in 19 of 27 patients.

The peak plasma concentrations, as well as the AUCs, increased in near proportion with increasing doses of MS-275 (Fig 4). The power model analysis indicated that the model poorly described the data, which estimates the parameter β was 0.517 ± 0.172 (R^2 = 0.323), while linear-regression analysis indicated near dose proportionality (R^2 = 0.556). The mean apparent CL/F of MS-275 was not significantly dependent on drug dose (P = .071) and the estimated $T_{1/2}$ was dose independent (P = .652). A preliminary analysis of pharmacokinetic-pharmacodynamic relationships for MS-275 suggests that drug exposure is significantly higher in patients experiencing DLTs (mean AUC, 517 ± 276 ng·h/mL, n = 4) compared with patients that had no DLT (280 ± 121 ng·h/mL, n = 23; P = .0477; Fig 5).

Analysis of PBMC Histone H3 Acetylation

Incubation of healthy donor PBMCs with MS-275 in vitro induced hyperacetylation of histone H3 (Fig 6A) in a concentration-dependent manner. Assayed at predosing and several time points postdosing, histone H3 hyperacetylation immunoflourescence images in PBMCs of two patients is shown (Fig 6A). The histone hyperacetylation quantified level was graphed for several patients (Figs 6B and C). The interpatient variability in histone hyperacetylation kinetics and intensities were apparent, as shown (Fig 6B: n = 7, 2 mg/m^2 and 4 mg/m^2; Fig 6C: n = 5, 10 mg/m^2). With limited sample size, there was no significant correlation between the AUC, AUC/dose, CL/F, C_{max}, C_{max}/dose, and the normalized change in histone H3 acetylation at 24 hours after the initial dose (data not shown). Histone hyperacetylation occurs at doses well below 10 mg/m^2, suggesting an optimal biologic effective dose may be much lower than the MTD defined by clinical toxicity.

DISCUSSION

To date, three subclasses of HDACs have been recognized. Class I, yeast RPD3 homologs (49-60 kD) include HDAC1,

Table 6. MS-275 Pharmacokinetic Parameters

Dose (mg/m^2)	No. of Patients	C_{max} (ng/mL) Mean	C_{max} SD	AUC (ng·h/mL) Mean	AUC SD	CL/F (L/h/m^2) Mean	CL/F SD	$t_{1/2}$ (hours) Mean	$t_{1/2}$ SD	T_{max} Median	T_{max} Range
2	3	1.72	0.23	196.26	104.5	13.77	10.27	80.20	48.68	6	2−24
4	3	4.84	1.10	391.68	150.71	11.33	4.57	50.51	12.96	6	2−36
6	6	9.59	4.57	492.81	177.77	13.18	3.43	52.78	20.25	2	2−60
8	5	15.49	11.65	357.71	38.14	22.58	2.71	39.73	15.23	2	0.5−24
10	6	45.07	59.34	528.87	170.57	20.50	5.99	51.58	10.49	1.5	0.5−2
12	4	131.63	128.3	680.16	262.0	19.85	8.01	45.00	6.53	0.5	0.5−2
Grand mean						17.40	6.75*	51.74	21.55†		
Grand median										1.75	0.5−60

*P value for Kruskall-Wallis test (P = .071)
†P value for Kruskall-Wallis test (P = .652)

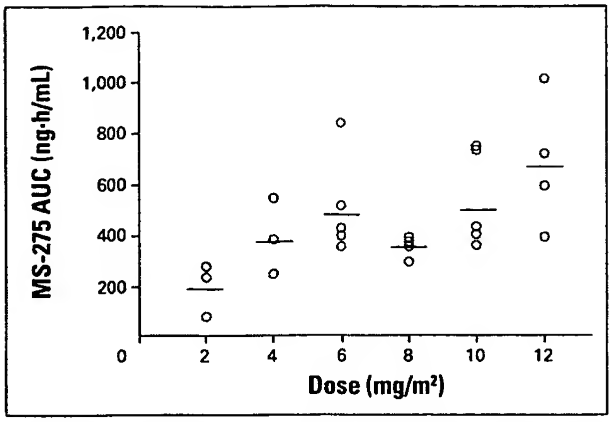

Fig 4. Effect of MS-275 dose on the area under the curve (AUC) at dose levels ranging from 2 to 12 mg/m^2 (n = 27). Each symbol represents data from an individual patient. Horizontal lines indicate the mean value for each dose group.

HDAC2, HDAC3, and HDAC8. Class II, yeast HDA1 homologs (> 100 kD) include HDAC4 (HDAC-A), HDAC5 (mHDA1), HDAC6 (mHDAC2), and HDAC7.[3,13-20] Class III are Sirt 1-7 and HDAC 11.[21] Different HDACs have been shown to associate with distinct transcriptional regulatory complexes[22-24] and different heterochromatic environments.[25] Nonhistone proteins, including cell structure elements (tubulin, HSP90), activators (p53, GATA-1), and transcription factors (TFIIE, TFIIF), were reported to be acetylated by histone acetyltransferases, suggesting that HDACs may regulate gene expression by deacetylation of nonhistone proteins.[26-29] HDACs may also participate in cell-cycle regulation, since Rb/E2F-mediated transcriptional repression involves recruitment of HDAC1 or HDAC2 by Rb.[30,31] HDIs present an exciting, novel approach for cancer therapy. They may augment gene-regulatory effects of coadministered DNA methyltransferase inhibitors.[33] Therefore, understanding HDI pharmacologic profiles as single agents is a prelude to constructing anticancer regimens to maximize gene expression modulation.

Several classes of HDIs have been identified: (1) short-chain fatty acids—butyrates[33,34]; (2) hydroxamic acids—trichostatin A,[34,35] suberoylanilide hydroxamic acid (SAHA),[2] and oxamflatin[36]; (3) cyclic tetrapeptides containing a 2-amino-8-oxo-9, 10-epoxy-decanoyl (AOE) moiety—trapoxin A[37]; (4) cyclic peptides not containing the AOE moiety—depsipeptide and apicidin[38,39]; and (5) pyridyl carbamates—MS-275.[5] A number of HDIs induce differentiation, growth arrest, and/or apoptosis of tumor cells in vitro.[5,30-41] Some were able to inhibit growth of cancer cells in animal models.[5,42-46] A smaller number of these may be less toxic to the host and able to target tumors selectively.[5,47,48] Different HDIs appear to inhibit different HDAC subgroups. MS-275 inhibits HDAC 1, 3, 4, and 10.[49] None of the HDIs recognized to date are known to inhibit class III HDACs.[49]

Our data indicate that MS-275 can be given safely on a q14-day schedule, but not on a daily schedule in the dose range explored. Most frequent toxicities, including DLTs, were fatigue and gastrointestinal symptoms of nausea, vomiting, and anorexia for the q14-day schedule. Myelosuppression became apparent among cumulative adverse events related to MS-275. Unlike the daily schedule, the q14-day schedule had neither symptomatic nor diagnostic cardiac adverse events observed. It is clear that for MS-275 used on a q14-day schedule, the low to median dose range of 2 to 4 mg/m^2 is well tolerated among patients. MTD of 10 mg/m^2 provided peak plasma concentrations on average exceeding 75 ng/mL. This is above concentrations required in vitro and in vivo to induce significant growth inhibition in many models for various primary human tumors.[5,50] Although objective responses were not observed, 15 patients had stable disease while on a q14-day schedule.

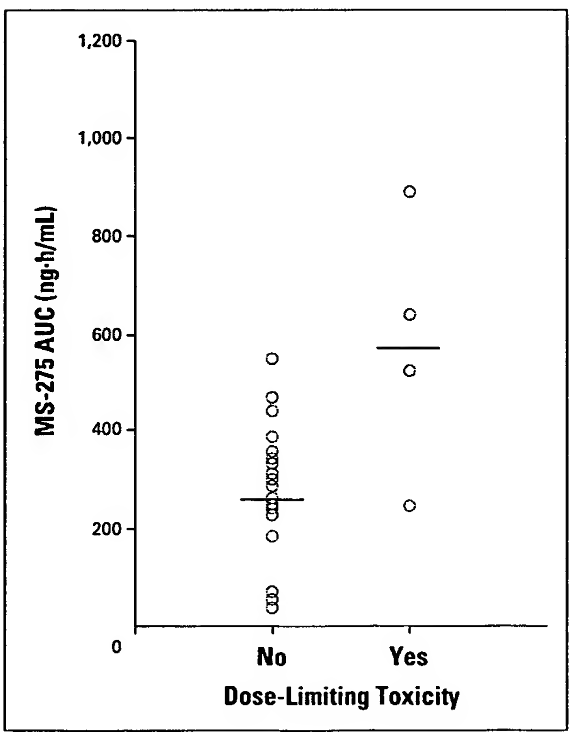

Fig 5. Comparative MS-275 area under the curve (AUC) from patients with and without a dose-limiting toxicity. Each symbol represents data from an individual patient.

Compared with the published data of other HDIs, neither grade 4 nonhematologic toxicities nor grade 2 or higher cardiac toxicities was observed on the q14-day schedule. Similarly, frequent nausea, vomiting, and dyspepsia were complications reported for sodium butyrate,[40,52] phenylbutyrate,[33,34,53] SAHA,[54] depsipeptide,[55] and tributyrin,[56,57] suggesting that the development of an oral HDAC inhibitor may be a challenge. The tolerable and reversible adverse event profile observed on a q14-day schedule does suggest that MS-275 might be a potentially

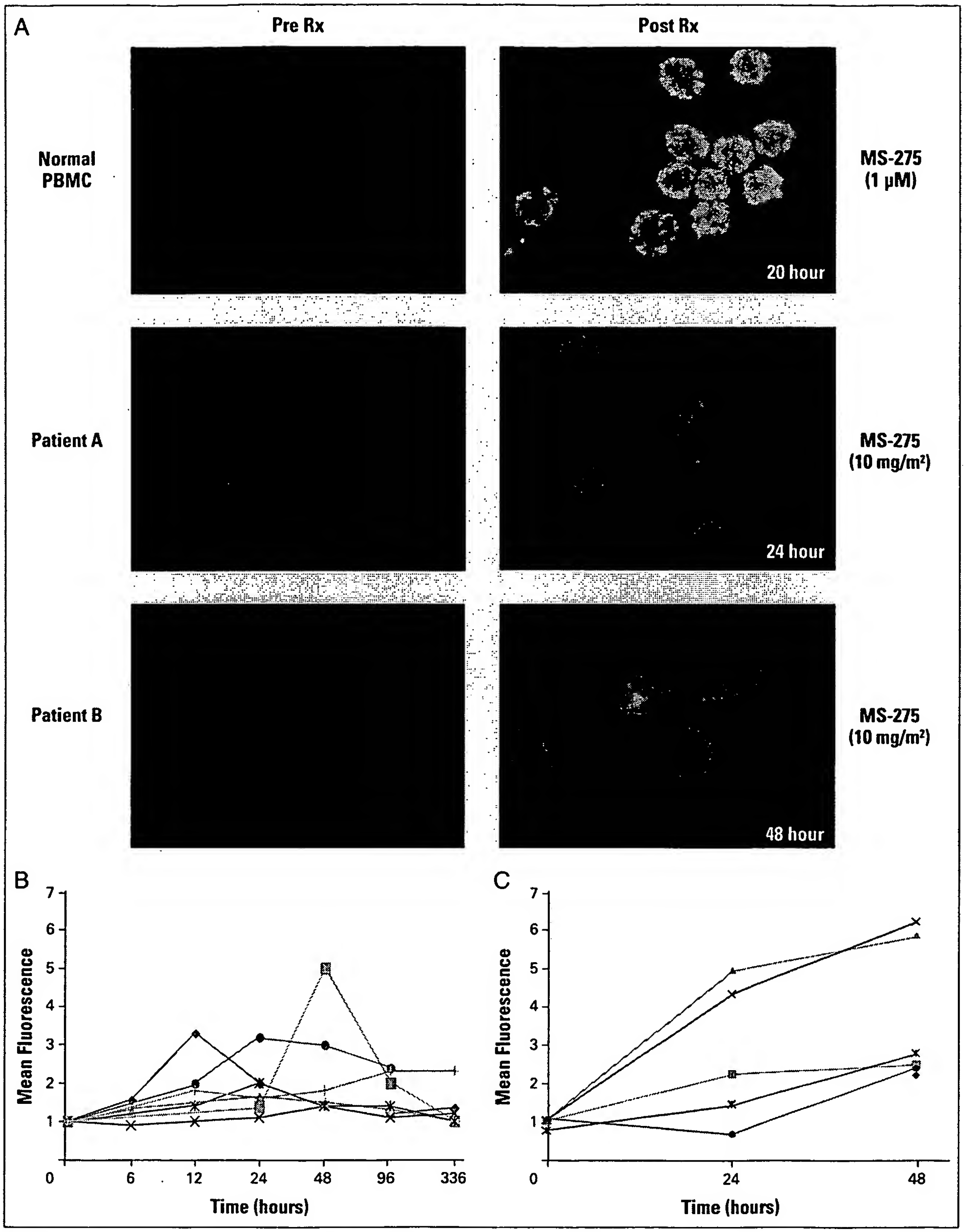

Fig 6. MS-275 induced histone H3 hyperacetylation. (A) Upper panel: healthy donor peripheral-blood mononuclear cells (PBMCs) incubated in vitro with MS-275 for 20 hours. Middle and lower panels: PBMCs from two patients treated with 10 mg/m^2 MS-275. (B) Mean fluorescence intensity of histone H3 acetylation in patients treated with 2 mg/m^2 MS-275; and (C) with 10 mg/m^2 MS-275.

well-tolerated chemotherapeutic agent. However, the q14-day schedule may not maintain a constant inhibition of HDAC activity. Presently, a weekly dosing schedule is being studied for tolerability and tumor response.

MS-275 displays a linear, dose-independent, pharmacokinetic behavior within the dose range studied (2 to 12 mg/m^2). Overall, drug absorption was rapid, and in some patients, the T_{max} was observed as early as 30 minutes, suggesting MS-275 might undergo rapid gastric absorption before reaching the small intestine. The disappearance of MS-275 was characterized by an apparent bi-exponential decline with a $T_{1/2}$ in plasma of approximately 50 hours, substantially longer than observed for MS-275 in laboratory animals (Schering AG, unpublished results). The basis for this long half-life in humans is possibly related to enterohepatic recirculation processes, suggested by the appearance of a second MS-275 peak around 24 to 48 hours after initial drug intake in several patients. Furthermore, the T_{max} observed at 24, 48, and 60 hours suggests a substantially longer normal gastrointestinal transit time. Any hypothetical recirculation is thus likely to mask the true disposition half-life of the free drug, as has been observed previously with many other agents.[58] Although other factors, including binding of the compound to plasma proteins such as human serum albumin and α_1-acid glycoprotein, may also influence the prolonged circulation of MS-275. We found that MS-275 is only $\approx$80% protein bound, and we did not find any greater binding affinity to albumin than to other proteins. Therefore, no significant clinical impact of protein binding on clearance is expected.

The observed variability in the pharmacokinetic behavior of MS-275, with an interpatient variability in the apparent CL/F of about 40%, is typical for cancer drugs administered orally.[59] Over the dose range studied, the MS-275 AUC demonstrated an apparent dose-independent behavior. Body-surface area correction did not account for the interpatient variability in clearance (38.8% *v* 39.5%), suggesting that body-surface area is not a significant predictor of oral MS-275 pharmacokinetics and that flat-dosing regimens might be applied without compromising overall safety profiles.

H3 acetylation in PBMCs provided a surrogate measure of HDAC inhibition after MS-275 administration. Our data demonstrate interpatient variability in the magnitude and kinetics of histone H3 hyperacetylation. Although MS-275 can induce histone H3 hyperacetylation in PBMCs in vivo, it is not clear whether histone H3 hyperacetylation is the most biologically relevant end point, nor is it known to what extent PBMCs reflect the MS-275 response in tumor cells in vivo. These should continue to be examined in relation to MS-275 and other clinically-relevant HCIs.

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Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Consultant: Edward A. Sausville, Schering AG; Research Funding: Jane B. Trepel, Schering AG. For a detailed description of these categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section of Information for Contributors found in the front of every issue.

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